PROTOPLASMIC MOVEMENT IN THE FORAMINIFERAN, ALLOGROMIA LATICOLLARIS; AND A THEORY OF ITS MECHANISM

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Protoplasmic streaming in reticulopodia has been described by numerous investigators, most of whom have commented on what an impressive phenomenon it is. One of the most detailed but condensed descriptions is that of Leidy (1879) who stated (pp. 279–280):

"In the emission of the pseudopodal filaments of *Gromia terricola,* the protoplasm pours from the mouth of the shell in a slow manner, and gradually envelopes the body. . . .From the protoplasmic envelope delicate streams extend outwardly, at first emanating from the front; they more or less rapidly multiply and radiate in all directions. Gradually extending, they fork into branches of the utmost tenuity. Contiguous branches freely join or anastomose with one another, and thus establish an intricate net, which in its full extent covers an area upward of four times the diameter of that of the body of *Gromia.* The pseudopodal net incessantly changes, putting forth new branches in any position, while others are withdrawn, diminishing and disappearing in one spot, while it spreads and becomes more complex in another spot.

"*Gromia terricola,* with its pseudopodal net fully spread, like its near relatives, reminds one of a spider occupying the center of a circular web. If we imagine every thread of the latter to be a living extension of the animal under the same control as its limbs, the spider would be a nearer likeness to the *Gromia.* Over each and every thread of the pseudopodal net, *Gromia* has a complete control as if the threads were permanently differentiated limbs acted on by particular muscles, and directed in their movements by nervous agency. Threads dissolve their connection and are withdrawn; new ones are formed and establish other connections; they bend; they contract into a spiral; they occasionally move like the lashing of a whip, and indeed produce almost every conceivable variety of motion. Not infrequently spindle-like accumulations of protoplasm occur in the course of the pseudopodal threads. Sometimes, through the conjunction and spreading of several of the latter together, islet-like expansions occur; and become centres of secondary nets.

"The pseudopodal extensions of *Gromia* consist of pale granular protoplasm with coarser and more defined granules. The latter are observed to be in incessant motion along the course of the threads, flowing in opposite directions in all except those of greatest delicacy."

Another excellent description of foraminiferan movement was provided by Jepps (1942) for *Polystomella.* She described more or less the same activity described by Leidy for *Gromia.* In regard to the pseudopodia she states (p. 624) that they "wave about like minute feelers, bending, undulating, quivering, and

putting out side branches which meet and fuse and so establish the reticulum". . . and that (p. 625) "The pseudopodia show a fairly high degree of stiffness; they extend in a straight line as a rule, and may stretch unsupported through the water for a distance at least two to three times as great as the shell diameter." (The greatest shell diameter she mentioned was about 1.8 mm.)

All of the above statements of Leidy and Jepps concerning streaming in *Gromia* and *Polystomella* are equally true of streaming in *Allogromia laticollaris,* and presumably apply to most, if not all, organisms with reticulopodial nets, with the exception that some species have been described as being much more active than others, but with no real differences described in the general type of protoplasmic activity, sometimes described as "filament streaming" (Fädchenströmung, Engelmann, 1879).

It has been recognized that the theory of flow caused by differential pressure in the plasmasol, which is so well accepted for *Amoeba proteus* and related genera and for Mycetozoa (reviews, Seifriz, 1942; De Bruyn, 1947; Bovee, 1952; Noland, 1957; also Kamaiya and Kurodo, 1958), would not explain the streaming of Foraminifera. This was pointed out clearly by Sandon (1934) who recognized the significance of the fact that there is no tube of plasmagel and no evidence of sol-gel reversibility in the active pseudopodia, and stated that it was time for another explanation to be developed. Considerable doubt concerning applicability of the pressure differential theory was also expressed by Noland (1957). So far no detailed alternative theory has been proposed to explain the streaming in Foraminifera, although the direction that such a theory should take was clearly pointed out by Noland (see below).

The purpose of the present paper is 1) to extend the observations of Leidy, Jepps, and others on movement in reticulopodial nets, 2) to postulate an active shearing mechanism for this type of movement, based on observations by the present authors and by previous investigators and on recently discovered facts concerning the mechanism of muscular contraction, and 3) to discuss briefly the taxonomic implications of the existence of two basic types of protoplasmic movement in the Sarcodina.

These two basic types of protoplasmic movement are: a) flow of plasmasol caused by differential pressure, which in turn is caused by contraction of a plasmagel cortex, and b) flow presumed to be caused by the newly postulated active shearing force between two adjacent oppositely moving gel-like filaments of protoplasm in the same pseudopod and in the absence of a typical plasmagel cortex.

Allogromia laticollaris was described by Arnold (1948) who has studied its movement and dispersal (Arnold, 1953), variation and isomorphism (Arnold, 1954), and life history and cytology (Arnold, 1955). Arnold's published studies on movement have included changes in location of the organism as a function of time and in relation to other organisms and in relation to environmental influences, but do not include a study of movement in the sense of protoplasmic flow and the mechanism of flow, as used in the present paper.

Material and Methods

Allogromia laticollaris, originally described from Florida (Arnold, 1948), is a common foraminiferan of the sea coasts of the United States. It is a large

organism, possessing a globular test with an average diameter of 200 to 400 microns, and a reticulopodial net of several times this diameter. It has been maintained continually in laboratory culture by Dr. Zach Arnold, who has kindly provided us with the ancestors of the organisms used in this work. The only requirements for prolific culture are sea water, nitrate and phosphate, some soil humus, a light source to permit growth of algae used as food, and containers, such as finger bowls. *Allogromia* is tolerant of heat and will reproduce within the range of 15 to 34° C.

Observations were made with the aid of ordinary bright field microscopes, a Zeiss inverted microscope with phase and bright field equipment, and a Leitz variable phase microscope, which also permitted dark field observations.

Micrurgical experiments were performed on organisms on open slides in a drop of sea water surrounded by a vaseline ring. Coverslips were added later for critical microscopic observations.

Results

1. General protoplasmic arrangement

The protoplasm of *Allogromia,* like that of all Foraminifera, lies 1) within the test, 2) around the outside of the test so that the test is more or less internal, and 3) in a network of pseudopodia, usually called reticulopodia, which may and usually do fuse peripherally to form complicated anastomoses, with numerous nodes, of various and continually varying sizes, all of which results in the formation of a reticulum, sometimes of very great complexity.

The following discussion applies specifically to the reticulopodia of medium or small diameter, *i.e.,* under 5 μ. Near the body of the organism some of the pseudopodia are larger, but at a short distance from the body those over 10 μ are rare. It appears as if some of the larger pseudopodia are fundamentally bundles of the smaller ones. Some of the following statements, *e.g.,* those concerning absence of a non-moving central core, and possible absence of a cell membrane, do not necessarily apply to the pseudopodia of larger diameter and definitely do not apply to the main body of protoplasm or even to the larger nodes of the reticulum.

Attachment to the substratum occurs in some of the more peripheral nodes of the reticulum, and presumably in some of the small peripheral masses of protoplasm sometimes found near the ends of the pseudopodia and which do not have side branches of reticulopodia, and sometimes under the main body of the organism. The active portions of pseudopodia under 5 μ are not attached directly to the substratum for much, if any, of their length, and many of them certainly are not attached to the substratum at all, except indirectly through the nodes or main body of the animal.

Branching and rebranching may occur throughout the length of the reticulopodia, *i.e.,* for several millimeters or more. However, a very high degree of branching occurs at the region where the mass of protoplasm merges from the opening in the test, so that the pseudopodia are seldom more than 10 μ in diameter at the base as they emerge from the general protoplasmic mass. In the early stages of emergence or the later stages of withdrawal the appearance of the numerous pseudopods sometimes resembles a tuft of brush bristles being pushed free end

foremost out from the body or being drawn into the body. A similar description is given by Doflein (1916) for *Gromia*.

2. Protoplasmic or filament streaming

Allogromia is able to extend a network of radial granular reticulopodia from its test as far as 15 millimeters in a circular pattern into its environment. In addition to the radial pseudopodia there are pseudopodia which form cross-connections between one radial pseudopodium and another. Individual pseudopodia have an average diameter of 2–5 μ, but some have a diameter of considerably less than 1 μ. Structure of a small branched pseudopodium is shown in Figure 1.

Streaming can be determined by observing the movement of granules. Previous investigators of the Foraminifera have pointed out that streaming is usually in two directions simultaneously in the same pseudopodium. One important point is that in our observations we have found that streaming is *always in two directions simultaneously* in every pseudopod as shown in Figure 2. In radial pseudopods one stream goes *toward* the body and the other *away* from the body, and in pseudopods that form cross-connections in the reticulum, each stream goes in the direction opposite from the other. We have never been able to observe streaming in one direction only. In certain of the smaller reticulopods it sometimes may appear superficially that movement is unidirectional because one stream may be out of focus or has fewer granules. However, upon careful focussing with bright field objectives and more easily with phase objectives we have *always* been able to find movement in the opposite direction even in the finest pseudopods. This is an observation that has great theoretical importance as far as the proposed mechanism of filament streaming is concerned.

In the medium sized and smaller pseudopodia of *Allogromia*, the protoplasmic material consists of two parts, each more or less the shape of a semi-cylinder, but also possibly flattened. In radial pseudopodia one semi-cylindrical portion is streaming in the outward or distal direction and the other semi-cylindrical portion is streaming in the inward or basal direction. We have not been able to detect a gel tube in any of the pseudopodia, even in those of large diameters. Neither is it possible to see the line of demarcation between the two oppositely moving layers.

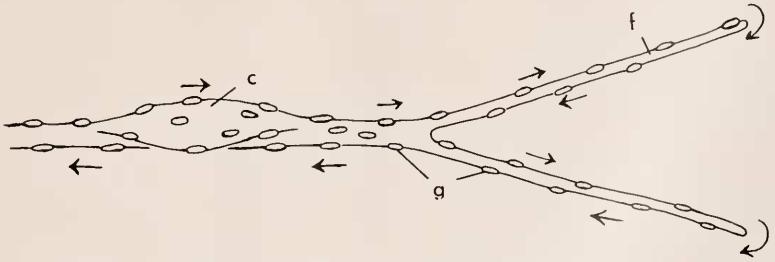

FIGURE 1. The general shape and structure of the distal portion of one of the finer pseudopodia, with a single bifurcation into branches about one micron in diameter. Arrows show movement of the granules (g), and of a small cytoplasmic mass (c), all of which are attached to the actively moving filament (f).

FIGURE 2. Anastomoses of three reticulopodia. Body of organism at left. Arrows show direction of streaming. Node (n) mentioned in text.

Reticulopodia are extended by a greater flow in the outward direction and at least usually are retracted by a greater flow in the inward direction. However, by simple visual observation it is not possible to say definitely whether greater flow is obtained by greater velocity or by greater cross-sectional area in one of the two directions.

The reticulopods are capable of great activity. They can bend and twist or even move laterally as they are extended; they can split, forming Y junctions, with base toward the body, and can anastomose, forming inverted Y junctions, with the base toward the periphery. One pseudopodium may split to form two pseudopodia, which may be parallel to or divergent from each other, with active two-way streaming in both. Also, new side projections or branches may be pushed out from a pseudopodium, and these projections are sometimes *carried* by the protoplasmic stream, and always exhibit two-way internal streaming themselves. The simple Y junctions can migrate along the pseudopodium in either direction and combine with other junctions to form X's and more complex types of junctions. Activity, always meaning double streaming, regardless of whatever else might be included, is at least almost continuous, and no pseudopodia appear to be in a condition of rest; those that are not streaming are invariably moribund. This constant state of activity, with continual splitting and anastomosing of pseudopodia, with bending, twisting, and lateral movement, can readily give the teleological impression, mentioned by Leidy (1879) that protoplasmic flow is under a most delicate if not deliberate central control, of the organism.

It is assumed that the streaming protoplasm of the reticulopodia is in a gel rather than a sol state. This assumption arises from the following observations:

1) Pseudopodial extensions only a few microns in diameter may be extended at almost any angle into the medium, for at least hundreds of microns, in a relatively straight line. They are more numerous along or near the substratum, but they are by no means limited to the substratum, and they certainly are not necessarily attached to the substratum.

2) The pseudopodia are not readily bent or shoved aside when bumped by ciliates, microcrustacea, or other swimming organisms, and exhibit a certain degree of rigidity.

3) The granules in the pseudopodia of small diameter, at least under simple visual observation (and with only occasional exceptions, mentioned below), seem to move in the stream without changing their relative positions and at least about the same distance apart.

The terms "gel" and "sol" are relative ones used to describe different ranges of viscosity. Consequently, the line of demarcation between them is not a sharp one. As used here, the word "gel" denotes a viscosity high enough to permit retention of form under a considerable degree of stress, for example, under the conditions mentioned above. In order to explain the observed phenomena the assumption of a considerable degree of rigidity seems necessary, and this degree of rigidity seems far in excess of that of the sol and comparable to that of the gel of *Amoeba proteus*. We have planned a cinephotomicrographic analysis in order to elucidate this point. The simple criterion of making an estimate of the degree of Brownian movement is not applicable because of the continuous streaming. The usual methods of measuring viscosity cannot be used for the same reason.

Another important observation is that at least in all pseudopodia under 5 μ in diameter *all* of the visible granules are streaming. *There is no tube of gel.*

Neither is there any appreciable space, or any other evidence whatever, for a hyaline layer outside of the moving stream. The stream consists of a hyaline gel material to which the large granules are attached.

Furthermore, there is no evidence for a central core of refractive non-moving protoplasm (stereoplasm), even when the pseudopodia are observed with the aid of dark field and phase equipment. This observation is in agreement with those of Doflein (1916) on *Gromia dujardini* in which he was unable to find a central core, and are in contrast to the observations of Doflein (1916), Schmidt (1937), Jepps (1942) and others who have described central cores in other genera of Foraminifera, which are not so closely related to *Allogromia*.

The granules of the reticulopodia are 2 to 4 μ in diameter, which may be greater than the average diameter of the pseudopodium in which they are contained. Therefore, they seem to be attached to rather than contained within the clear streaming material which comprises the actively moving portion of the pseudopodia. Sometimes the granules traveling in one direction can be observed to bump into granules traveling in the opposite direction, and to hit with such force that they become detached and then attached to the oppositely directed thread, thereby reversing their direction of movement. Less vigorous bumping may result in a backward shift of the position of a granule in relation to others in the same stream, but without a shift into the opposite stream.

Frequently there are small masses of protoplasm, more or less spindle shaped, up to several times the diameter of a pseudopodium, which migrate along with

the protoplasmic stream, either outward eventually to fuse with one of the nodes and build up a secondary protoplasmic center from which more pseudopodia may radiate, or inward toward the body. This has been described by Leidy (quotation above) and others for other species, and is shown in text Figure 1 (C). Such spindles are few in newly formed reticula but may be numerous in older ones.

Cross-connections may remain more or less in one position or may move laterally, that is, basally or distally, depending upon whether both ends are involved with outgoing or with ingoing streams, or may be pulled diagonally, with one end moving basally and the other end distally, if connected to one outgoing and one ingoing stream. Lateral movement of cross-connections, either basally or distally, but usually basally, is very useful in the engulfment of food particles.

Streaming granules can be seen going through the nodes of the reticulum in definite pathways, so that a node with a dozen or more or even with only a few radiating pseudopodia (as in Figure 2, n) seems a jumble of moving granules. These bump into each other continually, and therefore may seem superficially to be merely undergoing Brownian movement. However, closer examination under high magnification reveals that the pathways are quite definite and that most of the granules are moving in single file. Furthermore, under dark field illumination it seems at times as if these pathways are traced by very fine clear fibers to which the granules are attached, exactly as in the very fine pseudopodia described above.

Likewise, near the base of the larger pseudopodia which have many branches, the streaming is in the form of many narrow pathways, lying side by side, some with granules moving single file and some obviously in multiple lines, some directed basally and some distally, but usually with the lines well mixed in arrangement and not completely segregated according to direction. Similarly the pseudopodia intermediate in diameter seem to be made up of the same paired filaments of each of its branches, so that it is entirely probable that all except the finest pseudopodia are fascicles of the finer units, often with a partial fusion of filaments moving in the same direction, but certainly often without a complete fusion. This lack of complete fusion can account for the existence of more than one speed of streaming, as sometimes seen in the pseudopodia of intermediate and of larger diameter.

For these reasons it seems as if the protoplasmic threads to which the single rows of granules are attached are continuous, both in at least some of the nodes of the reticulum and in the larger pseudopodia. Therefore, in a certain sense and to a very considerable degree the paired hyaline filaments of the finer pseudopodia may be considered the fundamental structural units of the reticulum.

These fundamental streaming units, considerably less than a micron in diameter, are optically homogeneous as viewed with bright field, phase, and dark field objectives. Except for the bumps on the pseudopodia caused by the presence of granules the pseudopodia seem to be of quite uniform diameter throughout their unbranched portions, but they can differ in diameter from each other and are different in diameter before and after branching. We assume that this means that two or more of the paired fundamental units are fused to form all but the finest of the pseudopodia. In the smallest filaments there are fewer granules, but these granules can be traced individually as they move completely to the tip of the smallest clear filament and then turn 180° around the tip and start back toward the base of the filament.

Streaming is about 8 to 15 μ per second under conditions of our observations, but the results of modifying these conditions have not been studied.

The above description of filament streaming applies not only to the large adult organisms, but also to the smaller specimens, and even to the smallest ameboid forms that we have identified in cultures. Presumably this includes most of the stages of the life cycle.

Occasionally the end of a reticulopod may be turned back upon itself by extraneous forces and then begin to roll up into a spiral so the general form of the pseudopodium resembles a more or less flattened coil, with each turn in close contact and presumably fused with the adjacent turns, and with two-way streaming continuing for a number of minutes. The coiling seems to result when the tip of a pseudopod is bent so that it comes in contact with the outgoing protoplasmic stream. Two-way streaming continues in all parts of the spiral, and the coil continues to increase in size as long as contact is maintained only with the outgoing stream. It is possible that this is what Leidy meant when he stated (quoted above) "they contract into a spiral." Coiling requires about a minute, and a few minutes later the coil degenerates into a simple protoplasmic mass and then develops new pseudopodia.

3. Flow on the reticulopodial surface

Streaming of foreign material can easily be demonstrated on the surface of reticulopodia in *Allogromia* by use of a dye, *e.g.*, Evans brilliant vital red, which is insoluble in sea water. The dye particles, which may be ten or more times the diameter of the pseudopodia, stick to the protoplasmic surface and *flow along with the protoplasm* (Fig. 3). Individual dye particles may stick to either the distally or the basally directed streams and therefore may pass each other going in opposite directions. The same phenomenon can be demonstrated less colorfully by means of particles of finely ground glass. This is apparently a non-specific reaction, and occurs normally with all materials (primarily algae) that serve as the food source for the organisms. Normally food particles are carried by the basally directed stream until engulfed by the main body of protoplasm or by the distally directed stream until engulfed by one of the major distal masses in the network or until it is redirected into a basally directed stream.

4. Movement of the entire organism

When the organism is moving there is no apparent contraction of the anterior pseudopodia, as is well known for other shelled rhizopods (*e.g.*, *Arcella*). The anterior pseudopodia, which apparently pull the body and test forward, continue to have a rapid, and perhaps have a more rapid, two-directional streaming while the body is moving. The most reasonable explanation seems to be that the distal portion of the reticulum is attached to the substrate, that the distally streaming protoplasm is actually pulling the body forward, and that the motive power is the same active shearing process responsible for the streaming. The possibility should not be overlooked that movement may also involve some type of rapid contraction of the larger pseudopodia, as mentioned by Doflein (1916), Schmidt (1937), Jepps

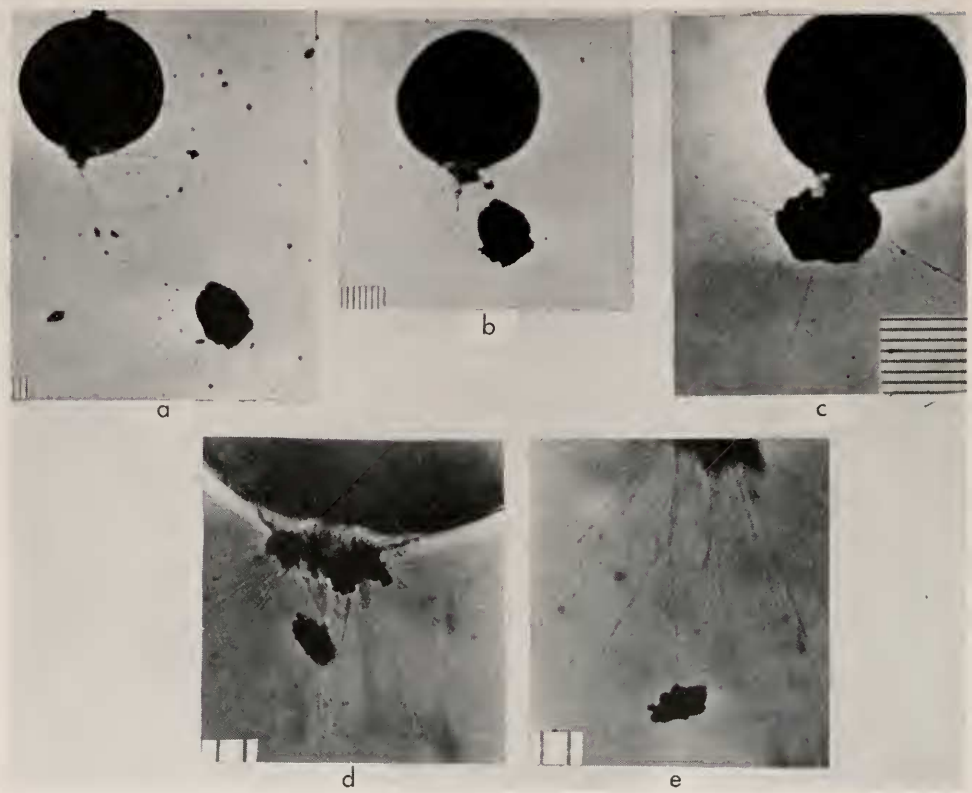

FIGURE 3. Photographs showing movement of dye particles attached to reticulopodia. *a*, Note large dye particles at lower right, and small ones scattered; *Allogromia* at upper left. *b*, Thirty minutes after *a*. Note movement of large particle toward opening in test of *Allogromia*. *c*, Thirty seconds after *b*. *d* and *e*, Dye particles moving along the pseudopodia. Note lack of engulfment of particle.

(1942), and others for other Foraminifera. However, we have not seen any movement which could be interpreted in this manner.

The tensile strength of active pseudopodia can be demonstrated by means of displacement with microneedles or movement of the medium. If a microneedle is entangled in some of the pseudopodia, the whole organism can be broken loose from the slide, leaving behind some fragments of protoplasm at the points of attachment, mostly in the peripheral portions of the net. Then the organism can be held by the microneedle attached to the pseudopodia while the slide is moved by the mechanical stage of the microscope. The pseudopodia which are not attached to the needle are dragged by the medium and trail as loose lines. When the direction of the slide is reversed the relative positions of the body and of the trailing pseudopodia are also reversed.

However, under these conditions the protoplasm in the pseudopodia attached to the needle, even if only a single pseudopodium, continues to flow, and in both directions.

5. Formation and behavior of protoplasmic fragments or satellites

We have confirmed the experiments of Grell (1956) that if the peripheral portion of a pseudopod of *Allogromia* is amputated, the fragment can fuse with the pseudopodial stump and again become part of the organism.

Furthermore, by repeated cutting of pseudopodia and removal of the main body of the organism we have been able to obtain small fragments of protoplasm. In small segments of a pseudopod, about 40 μ long, cut at both ends, two-way streaming was observed immediately after the cuts were made. This proves that the connection to the main body of the organism is not necessary for two-way streaming. However, such fragments soon become rounded, forming what we have termed "protoplasmic satellites." These satellites can persist for about forty minutes under conditions of our experiments. During this time they become stellate in appearance by extending several fine pseudopodia which are capable of bending, twisting, and anastomosing, and which exhibit two-way streaming. In small satellites most of the larger granules often remain in the central mass of the satellite and usually are rare in the pseudopodia (Fig. 4). The larger satellites have granular pseudopods. Satellites are capable of fusing with the parent organism and also with each other. Furthermore, upon disintegration the pseudopods of satellites have been seen to split into two filaments, free of granules.

Satellites also may be formed by rapidly crushing the organism between slide and coverslip. A rapid crushing causes most or all of the protoplasm of the body to dissolve in the sea water, but this does not necessarily result in solution of the uncrushed portion of the network. The larger nodes become the center of stellate protoplasmic masses, sometimes with dozens of radiating pseudopodia and numerous cross-connections, all of which exhibit two-way streaming. These may continue to stream for at least several hours. The nodes and the main body of these satellites also exhibit the definite granule pathways described for the intact organism. When the organism is crushed some of the pseudopodia may become completely isolated, and these also exhibit two-way streaming and may form small satellites similar to those obtained by micrurgical methods.

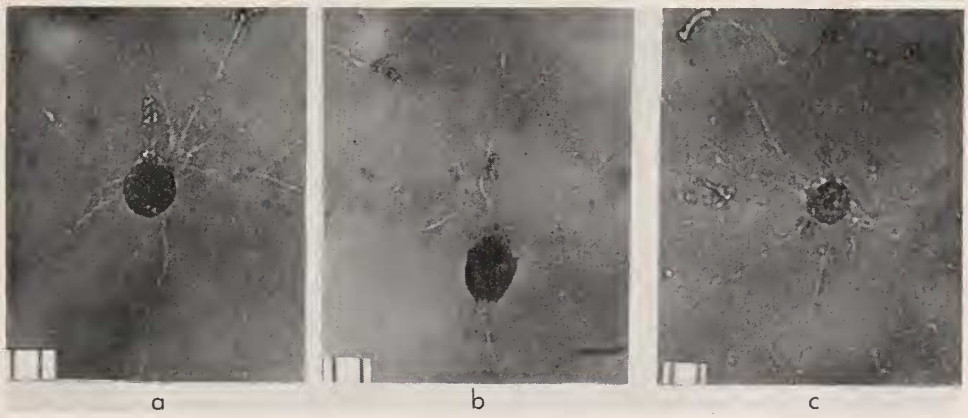

FIGURE 4, a, b, AND c. Protoplasmic satellites of *Allogromia*. Note anastomoses and orientation of pseudopods exactly as in intact organism.

Satellites which behave exactly as those described above can also be formed by quickly detaching the organism after it has extended pseudopods and attached them to the substrate, so that the attached ends remain attached to the substrate and become satellites.

In general, in so far as the size of the satellite permits, streaming in satellites is exactly the same as that in the intact organism.

Discussion

1. *Inadequacy of the pressure flow theory applied to Allogromia*

The idea that protoplasmic flow in *Allogromia* could be caused by differential pressure in a sol resulting from contraction of a partially surrounding gel tube does not seem to be in accord with the following facts:

1) Absence of a gel tube in the pseudopodia, and even the possible absence of a pseudopodial membrane (see below).

2) Presence of two-way streaming in all active pseudopodia, even those of smallest diameter, including all of the radial and all types of cross-connecting pseudopodia, both in the intact organism and in even the smallest protoplasmic satellites.

3) Presence of definite criss-crossing pathways for granules through nodes of the reticulum.

4) Presence of numerous parallel granule pathways in the larger pseudopodia, without segregation of pathways on the basis of direction, and without any evidence of gel tubes through which the granules could flow.

5) Presence of two-way streaming in freshly cut segments of pseudopodia.

6) Reversal of direction of granules at the tip of each pseudopodium.

Therefore, it is assumed that the theory of protoplasmic flow caused by differential pressure in a sol cannot apply to the protoplasmic flow of the reticulopodia of *Allogromia*. Likewise, it seems as if the pressure flow theory can not apply to the same general type of streaming in other Foraminifera, which has been described by other investigators. Therefore, a new theory is proposed.

2. *Theory of the mechanism of filament streaming in Allogromia*

The mechanism proposed to explain this type of movement is the existence of active shearing forces located in the reticulopodia between two paired filaments of protoplasmic gel, or rather between two portions of the same filament. These forces act longitudinally and oppositely and thereby produce the typical two-way streaming.

This is shown diagrammatically in Figure 5. In its simplest form the pseudopodium includes only two approximately semi-cylindrical, but possibly flattened, filaments of protoplasmic gel, labelled f_1 and f_2. Filament f_1 is the outgoing portion, and f_2 is the ingoing portion, as denoted by the large arrows. These are continuous at the tip of the pseudopodium and are therefore parts of the same filament. There may be some reorganization of the outgoing protoplasm at the tip before it becomes ingoing, but we have not been able to determine the degree of reorganization by simple visual methods.

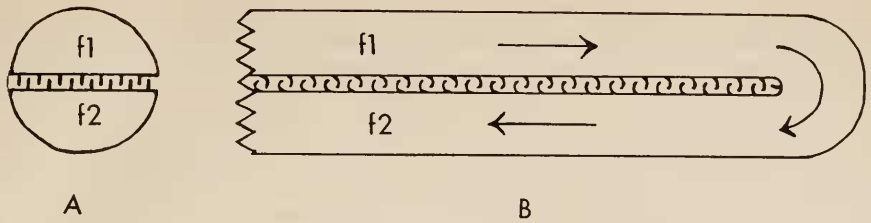

FIGURE 5. Arrangement of actively moving filaments (f_1 and f_2) of pseudopod. A, cross-section; B, side view. Direction of movement shown by arrows. The moving material is assumed in the diagram to be in the form of a semi-cylindrical filament, turned back upon itself at the tip, with the flat surfaces opposed. The shearing force is assumed to be between the adjacent surfaces and is designated by the short curved lines.

The small hook-like structures represent the active shearing mechanism, which at present is completely unknown. It must act in the direction indicated by the small arrows, and it must be capable of acting against the same mechanism on the opposite semi-cylinder. All other properties of the mechanism remain undetermined. As diagrammed here one could imagine the gel filament with its active mechanism to be similar to a millipede of relatively enormous and indeterminate length, folded back upon itself at the tip and with the legs of one portion of the body pushing against those of another portion throughout the length of the pseudopodium.

The basic idea is not new, but was proposed in somewhat different form by Noland (1957), in a general manner and without detailed application. In discussing the structure of protoplasm in *Amoeba proteus* Noland stated (p. 4), ". . . the endoplasmic molecules, some of them at least, must be quite linear in form. If one lets his imagination have free play he might compare the molecules of the plasmasol to a writhing mass of centipedes, each hanging on to his neighbors with a leg or two, but often losing hold and grasping any other one that comes with reach. Thus the whole mass, though moving, would maintain a certain coherence, so that a tug at one centipede would be communicated some distance into the mass." Furthermore, in expressing doubt that the pressure differential theory could be applied to reticulopodia, he stated (p. 6), "To revert to our centipede similes, what we need are molecules that can orient themselves in one direction and crawl forward on any solid surface, while others of the same sort crawl forward on the back of the first layer."

The present proposal, aside from substituting millipedes for centipedes, but otherwise continuing the simile, assumes that instead of one animal moving on the back of another, that the distally bound millipede is merely a more posterior segment of the basally bound millipede and that "they" are moving with the feet of one segment pushing backward against the feet of the other. One very important development since Noland wrote his paper on this subject is that it has now been definitely proven that in striated muscle we do have a mechanism that can "crawl" in the sense used by Noland, not on any solid surface, but on a highly specialized surface (review, A. F. Huxley, 1957). The existence of such a solid foundation changes what might have been considered a rather speculative hypothesis into a well founded theory, worthy of considerable development and investigation.

The completely unknown part of the mechanism is what the "millipede" uses for legs. In striated muscle the connections from the myosin fiber to the actin fiber, that is, the connections which actually exert the force involved in the sliding motion, may be seen with the aid of an electron microscope. It is also obvious from chemical and cytological studies that ATP is used by the sliding mechanism (review, A. F. Huxley, 1957). Otherwise the status of definite knowledge is not much more advanced for muscle than for reticulopodia; the actual mechanism of the "legs" is unknown in both. Furthermore, in reticulopodia we can observe this sliding of one fiber upon another continuously by means of an ordinary microscope for hours, or even for days, or as long as the patience of the observer persists.

In addition to the active shearing force there obviously must be some mechanism that holds the two active surfaces together and more or less in contact with each other. If the active force is transmitted through protein bridges similar to those which can be demonstrated in electron micrographs of striated muscle (H. E. Huxley and Hanson, 1955; A. F. Huxley, 1957), then the same bridges serve both purposes, and the two mechanisms are really two aspects of the same.

It is obvious that the word "streaming" when used in conjunction with the present theory of filament streaming is used in a very special sense, and only for historical reasons. It does not mean the streaming of a sol, as in *Amoeba* or in *Physarum,* or even in Foraminifera as assumed by previous investigators. It means the longitudinal movement of gel-like filaments, or threads, of protoplasm, carried along by active processes on the adjacent surfaces of the threads themselves, that is, on the surfaces between the two parallel threads which are "streaming" in opposite directions.

One unanswered question concerning which we have no evidence is whether the filaments of gel remain essentially as filaments when the material is all inside of the body, or whether they are in the form of undifferentiated protoplasm, which is re-formed into filaments when the pseudopodia are re-extended. The tuft-like appearance of many simultaneously emerging pseudopods might suggest that the structure is not completely destroyed within the body.

Another unanswered question is how the organism is able to extend or retract all pseudopodia simultaneously. Is there a central control of these movements? Perhaps, and perhaps not. However, if there is a central control, the present theory offers a very simple explanation. The outgoing thread of gel must be formed from, or at least released from, the body of the animal, and the ingoing thread must be incorporated into the body, either as a stored filament or as dedifferentiated protoplasm. Extension and retraction could be controlled very simply if there were control of either the release or the re-incorporation of the filaments, or of both of these processes.

One characteristic of reticulopodia which perhaps should be emphasized is that in at least all of the finer and possibly in all of the pseudopods there is no tube of plasmagel as in *Amoeba proteus* and in *Physarum.* This general characteristic has been noted by most of the earlier investigators (*e.g.,* Doflein, 1916; Schmidt, 1937; Jepps, 1942). The fact that a gel tube is absent is of great theoretical importance from the viewpoint of explaining the mechanism of movement. The absence of a gel tube definitely rules out the pressure differential theory, unless one assumes that the cell membrane is mechanically capable of performing the functions ordinarily

assigned to the tube. The question of the structure of the membrane and even the possibility of its non-existence on the reticulopodial surface is discussed below.

Leidy stated that streaming was always in two directions in all pseudopodia "except those of greatest delicacy." It is possible that he was not able to detect oppositely directed movement because of a scarcity of granules in the opposite stream. However, it is also possible that some of the "pseudopodia of greatest delicacy" are formed by splitting of an undirectional thread from a pseudopodium and that this thread is merely pushed passively into the medium. The present authors have seen a few temporary pseudopods which could be interpreted in this manner. However, such a thread, at least according to the present theory, could not develop into an active pseudopodium without developing a return flow.

The theory, as outlined above, will explain all of the facts as we know them, including the six items listed above (Discussion, section 1) which cannot be explained by the theory of differential pressure in a sol.

3. Application of the theory to other materials

It seems as if the present theory could apply in slightly modified form to the reticulopodia or rhizopodia which have a central core of stereoplasm and also to axopodia, which have a well defined axial filament. From one point of view the only change necessary is to assume that the active mechanism is capable of moving along the surface of the stereoplasm or the axoneme rather than only along the active surface of an oppositely directed filament. This is the equivalent of introducing Noland's idea of molecules crawling upon a solid surface. Another possibility is that the active shearing occurs not between the rheoplasm and the stereoplasm or axoneme, but between the outgoing rheoplasm and ingoing rheoplasm where they come in contact with each other, peripherally to the stereoplasm or axoneme. The central cores and the axonemes may serve architectural functions, but since they do not exist in *Allogromia* it is obvious that they are not necessary to explain either the stiffness or the streaming that exists in the reticulopodia of *Allogromia*.

Previous investigators (*e.g.*, Doflein, 1916) have pointed out that as the pseudopod is extended, more stereoplasm is added at the distal end of the core and that this must come from the rheoplasm. If so, and if the present theory also applies to species which have a central core, the core is composed merely of temporarily inactivated fibers of rheoplasm, that is, of the hyaline material without the attached granules.

Many of the stamen hair cells of plants have two-way streaming in fine threads of protoplasm which go across (that is, through) the cell vacuole (*e.g., Zebrina, Tradescantia*). In many instances it is obvious that streaming is in both directions. However, streaming sometimes appears to be undirectional because the cytoplasm from one direction has few granules and is therefore difficult to detect by observation. It seems in such cases that the observers (especially students in elementary classes) are often confused when a few large granules or even chloroplasts, are seen moving apparently upstream. According to the present theory, the granules are merely moving along in the colorless stream of a granular cytoplasm. The granules apparently take no active part in the streaming process, but purely a passive one, as in the Foraminifera.

The idea of an active shearing force is not limited to filament streaming but may also be applied to the contraction that occurs in the posterior end of *Amoeba proteus*. In various other ameboid organisms and in leucocytes, it seems quite definite that contraction of the protoplasm does occur (Mast, 1926; Lewis, 1931; review, De Bruyn, 1947), but the supposed contraction of the protein molecules of the gel, as proposed by Goldacre and Lorch (1950) is merely a theory based on the old idea that muscle contracts by a folding or a spiralling of linearly arranged elongated protein molecules. Allen (1955) sucked protoplasm of *A. proteus* into capillary tubes and then observed two-way streaming, during which each stream behaved as a structural unit, which could became subdivided to form narrower streams, so small as to contain only a single row of granules. This seems similar to the two-way streaming in the reticulopodia of *Allogromia*. If this type of streaming can occur in the normal endoplasm of *Amoeba* it could be the physical basis of the contractile process. The possibility of such an explanation is mentioned by Noland (1957, quotation above).

Another alternative to the folding mechanism proposed by Goldacre and Lorch (1950) is the limited folding or sliding-folding mechanism suggested earlier by Frey-Wyssling (1948). According to this suggestion the sliding is caused by a wave of limited folding which passes along an elongated molecule. It is really an explanation, without experimental evidence, of how a sliding or creeping of one molecule along another might occur.

The contraction of the transparent pseudopodia of *Arcella* might also involve an active shearing rather than a molecular shortening, but on this point there is no evidence whatsoever.

The idea of an active shearing force can be applied to cyclosis whenever it occurs, *e.g.*, in *Nitella, Chara, Elodea, Paramecium, Vorticella,* etc. The only assumptions needed are that the moving material has a high viscosity and that the active force is exerted tangentially on its outer surface by the inner surface of the fixed cortical gel, or that the force is exerted from the moving high viscosity sol or gel on to the fixed cortex. Similar assumptions can also explain the rotatory movements of fragments of cells of *Nitella* and *Chara* described by Yatsuyangi (1953a, 1953b).

This possibility is well demonstrated in the work of Jarosch (1958) who has succeeded in isolating filaments from the protoplasm of *Toyellopsis* (Characeae). Jarosch has shown that these fibers are actively motile because they produce parallel displacement forces, that they have an affinity for small microsomes (*cf.*, granules of *Allogromia*), that they fuse into thick bundles, that they have the consistency of a gel, and that they possess elasticity. In brief, Jarosch has described in the filaments of *Toyellopsis* exactly the properties needed to explain streaming in *Allogromia*. In an earlier note he also mentioned the possibility of applying a similar theory to ciliary movement and to movement in axopodia (Jarosch, 1957).

In summary, it seems as if we can postulate two major types of protoplasmic movement in the Sarcodina, and possibly only these two types for all protoplasmic movements. These are:

1) Flow of a sol caused by differential pressure as a result of contraction of a partially surrounding gel, and

2) Movement of gel, which in *Allogromia* (and probably in plant hair cells) is in the form of paired filaments of gel which move by means of active shearing

forces, acting oppositely and longitudinally along the adjacent surfaces between the threads. In reticulopodia of most other Foraminifera the active force of the filaments, instead of acting on the other filament, might act against the stereoplasm, or, in axopodia, against the axoneme. In cyclosis the force from one layer of gel may act against that of another layer of thick sol or gel, or vice versa.

Furthermore, we have the possibility that the gel contraction that causes pressure flow may involve an active shearing mechanism as the contractile process of the gel.

4. Taxonomic significance of the two types of protoplasmic streaming

Certainly, if we consider only the Sarcodina as a group, we can state that we have both pressure flow and filament streaming as the two types of protoplasmic movement. If we ignore the untested possibility that both of these might be fundamentally the same, and consider them to be distinct, we are faced with the very interesting question of how they are distributed taxonomically. If this is a fundamental difference in the type of movement, perhaps all Sarcodina which have only filament streaming should be placed in a separate group from those which exhibit only pressure streaming, and those which have both should be placed in an intermediate group.

If this were done we would have to consider organisms with filopodia, reticulopodia, rhizopodia, and axopodia more closely related to each other than to those which have lobopodia only. This would necessitate changes in the well known separation of the Sarcodina into Rhizopodea and Actinopodea, and a re-assortment of the organisms placed in the rhizopodean order Proteomyxida, most of which have filament streaming. Such a thorough reorganization does not seem justified at present. However, the lines of demarcation between the current orders of the Sarcodina are so far from being satisfactory that a reclassification may well be contemplated in the future when more information becomes available.

5. Nature of the reticulopodial surface

The nature of the protoplasmic surface of reticulopodia has been the subject of comment by various investigators. It is commonly agreed that most but not all objects normally brought into contact with the pseudopodia will stick, and furthermore, *those that stick will be carried along in or on the protoplasmic stream.* Arnold (1953) mentioned this fact in regard to the food material used by *Allogromia.* In our experiments the insoluble dye and the glass particles stuck and were carried in both directions by the protoplasmic streams. Some particles do not stick tight enough to be engulfed. For instance, Sandon (1934) cites the fact that flagellates often stick to foraminiferan pseudopodia, are carried for a considerable distance, and then break loose and swim away. Sandon interprets this as evidence of a tough protective pellicle over the pseudopod. However, it could better be interpreted as evidence that the membrane is either thin and delicate as well as sticky, or even non-existent.

If one assumes that the streaming protoplasm is actually a thread of gel, then there is no need of assuming any membrane whatever in order to explain the mechanics of streaming. In fact, the principal reason for assuming the existence

of a membrane is that to assume the opposite would be physiological heresy. It seems as if the assumption that a membrane does not exist is just as radical as the assumption of the existence of an active shearing force would have been a few years ago.

If we assume that a reticulopodial membrane does exist and that foreign particles which move with the flow are sticking to the membrane, then the membrane itself must move with the stream, as assumed by Sandon (1934). If so, then the portion of the membrane over the outgoing stream is moving oppositely from that over the ingoing stream, and the membrane, if it is to be considered a single membrane, must be sheared along two longitudinal lines, one on each side, where the circumferential margins of the oppositely moving streams are closest to each other. Therefore, along these two lines the membrane is continuously subject to longitudinal shearing and must be very highly labile; for all mechanical purposes such a membrane might as well not exist. The assumed membrane, then, as far as structure is concerned, becomes the membrane, not of the pseudopodium but of each protoplasmic stream, and it could well be merely the surface of the gel which makes up the moving protoplasmic thread.

On the other hand, if we assume that the foreign particles penetrate but are not completely covered by the membrane and stick to the moving gel thread, and that the membrane does not move, then the large particles, of a size, let us say, ten times the diameter of the pseudopodium, must split the membrane as it moves with the stream, and the membrane must be re-formed behind the particle. If we assume that these large particles upon contact with the gel are immediately covered by some sort of a membrane, then we must assume that the membrane is very rapidly formed at the anterior edge of the particle, and destroyed in the posterior edge of the particle, and this seems even more unlikly.

For these reasons it seems best to assume tentatively that the membrane of the small reticulopodia may be merely the surface precipitation membrane, of possibly merely the surface, of the moving thread of protoplasmic gel that constitutes the stream.

This tentative assumption, if made in addition to the theory of mechanism outlined above, results in perhaps the simplest overall concept of reticulopodia that is possible . . . merely two adjacent naked filaments of gel, or more exactly two parts of the same filament, pushing against each other longitudinally along their adjacent surfaces, with resultant two-way streaming. This concept may be oversimplified, but for the present there seems to be no reason for assuming without evidence the existence of any of the complicating structural considerations found in other material.

Summary

1. Protoplasmic streaming in the reticulopodia of *Allogromia laticollaris* is described. Streaming is always a two-directional movement of two threads of plasmagel which together with attached granules seem to make up the entire structure of the reticulopodia. There is no outer tube of gel, no central core of optically refractive material, and no space for an outer hyaline layer. This seems to be the simplest form of filament streaming known to exist.

2. It is proposed that the mechanism of filament streaming in *Allogromia* consists of active shearing or parallel displacement forces located between the adjacent surfaces of the two gel filaments, acting longitudinally and oppositely from one filament to the other so as to produce two-way streaming.

3. Possible applications of the theory of active shearing forces to protoplasmic movement in other materials are discussed.

4. It is suggested that in *Allogromia* the gel threads of the reticulopodia may not be covered by a typical cell membrane but by a surface precipitation membrane or that the membrane may be merely the surface of the gel filament itself.

5. The possible taxonomic significance of the existence of two major types of protoplasmic movements, namely, pressure flow and filament streaming, is discussed.

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